

# Ovarian Structure and Oogenesis of the South African Heel-walker *Karoophasma biedouwensis* (Insecta: Mantophasmatodea)

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## Abstract

Ovarian structure and oogenesis of the South African heel-walker *Karoophasma biedouwensis* were examined using light and transmission electron microscopies. The adult female has a pair of ovaries, each of which consisted of five or six ovarioles directly connected with a lateral oviduct. The ovariole was composed of an anterior terminal filament, a germarium and a vitellarium next to the lateral oviduct. The germarium was very short and contained a small number of oogonia, very young oocytes and remarkably flattened prefollicular cells. No intercellular bridges were found between the oogonia or the very young oocytes. In the vitellarium of the full-grown ovariole, there were seven to 14 developing oocytes arranged in a single file, in order of development. Each oocyte was surrounded by a follicular epithelium, and the proximal oocyte was chorionated. No nurse cells developed. The ovariole type of Mantophasmatodea is typical panoistic. The late previtellogenic oocytes were characterized by an abundance of mitochondria and fragments of endoplasmic reticulum, and by the presence of interdigitation of microvilli formed between the oocyte and follicular epithelium. We compared the ovarian structure and oogenesis of Mantophasmatodea with those of other orthopteroid orders, and discussed the affinity of Mantophasmatodea within orthopteroid orders.

## Introduction

The most recently recognized order Mantophasmatodea was named due to its superficial resemblance with two orthopteroid orders, Mantodea and Phasmatodea (Klass *et al.*, 2002). Both morphological and molecular evidences indicate that Mantophasmatodea belongs to an orthopteroid order (Klass *et al.*, 2002, 2003; Gullan and Cranston, 2005), but the sister group relationships of Mantophasmatodea with other orthopteroid orders remain unidentified (Klass *et al.*, 2002; Zompro *et al.*, 2002; Klass, 2004).

Insect ovariole analysis has been used to understand insect phylogeny (cf. Büning, 1998). In the present study, we examine the ovarian structure and oogenesis of the South African heel-walker *Karoophasma biedouwensis* (Mantophasmatodea) with special reference to ovariole type, and compare the results with those of other orthopteroid orders.

## Materials and Methods

Adult female *Karoophasma biedouwensis* were collected in Namaqualand (Western Cape Province, South Africa) in September 2003. The left and right ovaries were removed from their bodies in Karnovsky's fixative (2% paraformaldehyde +2.5% glutaraldehyde) buffered with 0.1 M HCl-sodium cacodylate and refixed with the same fixative. The ovaries were then post-fixed with 1% osmium tetroxide, dehydrated in a graded acetone series, embedded in water-miscible epoxy resin, Quetol 651 (Nissin EM), and cut into ultrathin sections. The sections were

double-stained with uranyl acetate and lead citrate and observed under a transmission electron microscope (JEOL JEM 1010) at 80 kV. Semi-thin sections 1  $\mu\text{m}$  in thickness were stained with toluidine blue O and observed under a light microscope.

## Results

### Ovarian structure

There were a pair of ovaries and lateral oviducts fused into a common oviduct in the abdomen of *Karoophasma biedouwensis*. The ovary consisted of five (or rarely six) ovarioles of the same oogenetic stage, connected serially on one side of the lateral oviduct, to make a comb-like alignment (Fig. 1). Each ovariole was composed of an anterior terminal filament, a very short germarium and a posterior vitellarium. In the full-grown ovariole, the vitellarium contained seven to 14 developing oocytes arranged in a single file. Each oocyte was surrounded by a follicular epithelium and the most proximal oocyte next to the oviduct was chorionated. No nurse cells developed. The number of the ovarioles including chorionated oocytes in paired ovaries corresponded to the number of mature eggs in an egg pod (10 to 12) reported by Tojo *et al.* (2004) and Machida *et al.* (2004). After the ovulation of mature oocytes into the lateral oviduct, the next oocyte became chorionated in each vitellarium.

Each ovariole was tightly covered with two layers of envelope: a thin inner layer of non-cellular tunica propria (*ca.* 0.25  $\mu\text{m}$  in thickness) and a thick outer layer of cellular tunica externa (*ca.* 5  $\mu\text{m}$  in thickness) (Fig. 2). The cytoplasm of the tunica externa cells was rich in mitochondria and rough endoplasmic reticulum (arrows in Fig. 2).

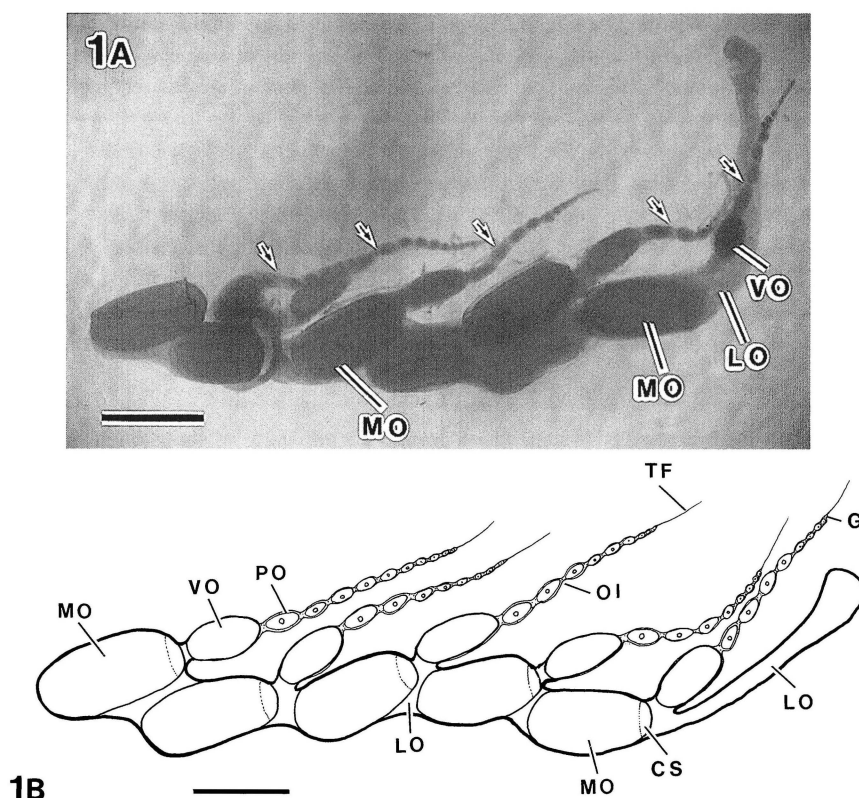


Fig. 1 Adult ovary of *Karoophasma biedouwensis*. A. Whole mount preparation of the ovary. B. Diagrammatic representation of the ovary. The ovary consists of five panoistic ovarioles (arrows). Mature chorionated oocytes (MO) have been ovulated into a lateral oviduct (LO). CS: cap structure, G: germarium, OI: ovariole, PO: previtellogenic oocyte, TF: terminal filament, VO: vitellogenic oocyte. Scales = 2 mm.

### *Oogenesis (previtellogenic oocyte growth)*

The germarium of adult ovary of *Karoophasma biedouwensis* contained a small number of oogonia, very young oocytes, remarkably flattened prefollicular cells and more or less cuboidal inner sheath cells. No intercellular bridges were found between the oogonia or the very young oocytes (Fig. 3). In the cytoplasm of oogonia and very young oocytes in the germarium, no definite organelles developed except for a small number of mitochondria. No septum separated between the germarium and terminal filament.

The early to middle previtellogenic oocytes were surrounded by a thin follicular epithelium (*ca.* 1  $\mu\text{m}$  in thickness) (Fig. 2). Several mitochondria and a small amount of rough endoplasmic reticulum appeared in the cytoplasm of early previtellogenic oocytes. In the cytoplasm around the nucleus of the middle previtellogenic oocytes, there were several masses of so-called nuage material, abundant variously shaped mitochondria, rough endoplasmic reticulum, Golgi apparatuses and small vesicles derived from the endoplasmic reticulum (Fig. 4). In the periplasm, however, few organelles were found at this stage. In the cytoplasm of the follicular cells, only a few mitochondria and a small amount of rough endoplasmic reticulum were observed.

The late previtellogenic oocytes were characterized by the presence of a complicated interdigitation of microvilli formed between the oocyte and its follicular epithelium (Fig. 5). There were a large amount of fragments of endoplasmic reticulum throughout the ooplasm and numerous pinosomes (arrowheads in Fig. 5) in the periplasm, but few other organelles. The follicular cells remarkably grew into cuboidal (4–8  $\mu\text{m}$  in height). The nuclei of follicular cells also grew and some of them were deeply hollowed in one side into C-shaped (arrows in Fig. 5). A number of mitochondria, a small amount of rough endoplasmic reticulum and a small number of middle-sized vacuoles were observed in the cytoplasm of follicular cells. Then, vitellogenesis occurs in the proximal oocyte of each ovariole, and will be described in a future paper.

## Discussion

### *Mantophasmatodean ovariole type*

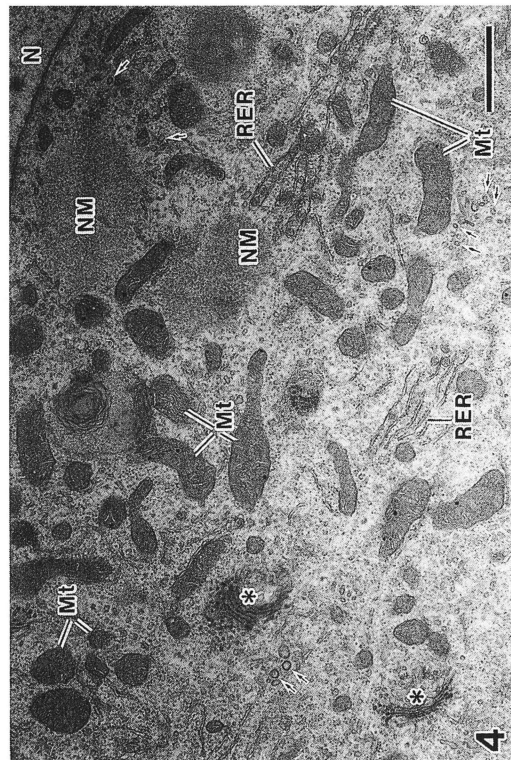
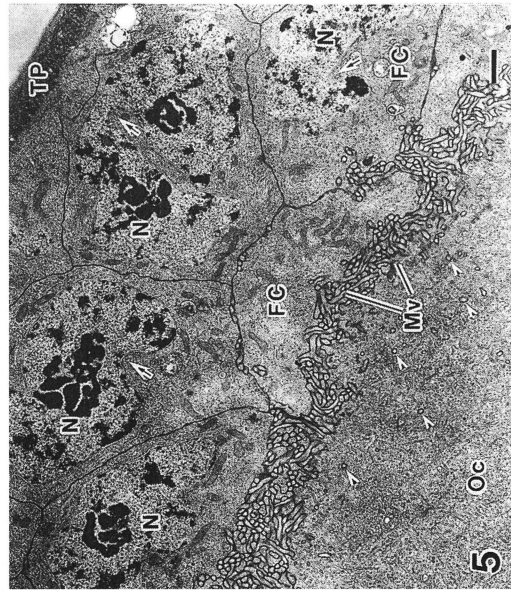
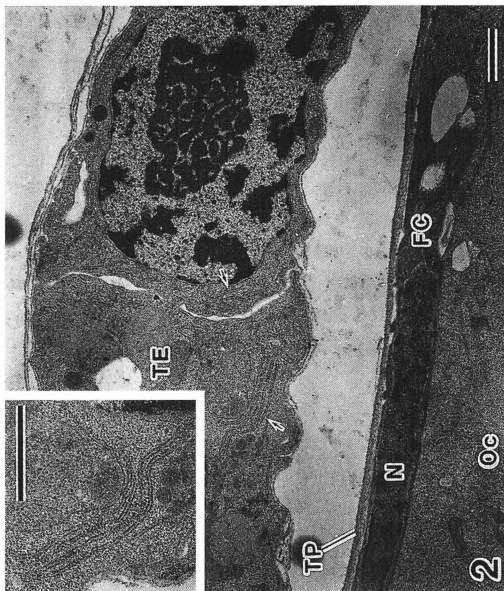
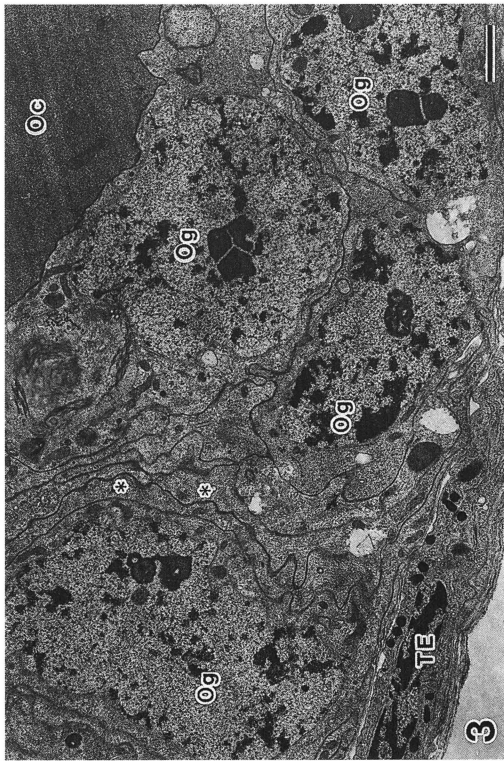
In the present study, no intercellular bridges were found between young germ cells in the germarium, and no nurse cells developed in either the germarium or vitellarium of *Karoophasma biedouwensis*. Therefore, we conclude that the ovariole type of *K. biedouwensis* is typical panoistic as most other orthopteroids (cf. Table 1). Moreover, in another mantophasmatodean *Sclerophasma paresisensis*, collected in Namibia in 2002, the light microscopy revealed that the developing oocytes arranged in a single file in the vitellarium were accompanied by no nurse cells as in *K. biedouwensis* (Tsutsumi, unpublished). Possibly the fact may help to extend our conclusion from *K. biedouwensis* to Mantophasmatodea.

### *Comparison with other orthopteroid orders*

Table 1 summarizes the characteristics of ovarioles in *Karoophasma biedouwensis* (Mantophasmatodea) and other orthopteroid orders. Most orthopteroids including *K. biedouwensis* have ovarioles with typical panoistic features as mentioned above. In Plecoptera, the intercellular bridges connecting oogonia or very young oocytes have been found in their panoistic ovarioles (Gottanka and Büning, 1990). In other orthopteroid orders such as Grylloblattodea, Blattodea, Mantodea, Isoptera, Phasmatodea, Embioptera, Orthoptera and Zoraptera, the panoistic ovariole features have been described (cf. Büning, 1994). In Dermaptera, the polytrophic meroistic ovarioles have been reported (Yamauchi and Yoshitake, 1982).

The panoistic ovarioles of *Karoophasma biedouwensis* are connected serially with the lateral oviduct without any connecting structure such as ovariole stalks (pedicels). This comb-like alignment of the ovarioles on the lateral oviduct is found in most orthopteroids. Comb-like alignment, probably derived from metameric alignment of the ovarioles, is considered to be the primary type of the ovariole alignment in insects (Biliński and Szklarzewicz, 1992; Büning, 1994), whereas bunchy alignment, in which the ovarioles are connected concentrically with an oviductal calyx *via* the ovariole stalks, is generally seen in more derivative order such as Hemiptera, Thysanoptera and holometabolous orders (Haga, 1985; Büning, 1994; Tsutsumi, 1996). Among the orthopteroids, Blattodea, Mantodea, Isoptera and some orthopterans have the ovarioles of bunchy alignment (Table 1). In this respect, Mantophasmatodea and Mantodea, which is one of the suggested sister groups of Mantophasmatodea (Dallai *et al.*, 2003), are clearly different.

Most species of orthopteroid orders have a relatively small number of ovarioles, although some remarkable



Figures 2-5

variations in number are seen even in the same order (Table 1). The number of ovarioles per ovary, however, may also depend on other factors such as the body size, relative size of the reproductive system, and the fecundity in a reproductive season. In most orthopteroid orders, each ovariole is surrounded by an inner tunica propria and an outer tunica externa, although the stoneflies have no tunica externa (Büning, 1998). The very thick tunica externa of *Karoophasma biedouwensis* may be a feature adapted to their dry habitat.

Phasmatodea is suggested as another candidate for the sister group of Mantophasmatodea among the orthopteroids (Klass *et al.*, 2002). Their previtellogenic ooplasm is characterized by the numerous multivesicular bodies containing an electron-dense matrix in various phases of condensation and a large amount of Golgi apparatuses especially in the periplasm (Giorgi *et al.*, 1993). In the previtellogenic ooplasm of *Karoophasma biedouwensis*, however, there are only a few Golgi apparatuses around the nucleus, and no multivesicular bodies. These organelles are closely related to vitellogenesis, and therefore they are significant features of the late previtellogenic oocytes in panoistic ovarioles, particularly with regard to the kinds of organelles and to the period they begin to emerge in the ooplasm.

In the follicular cells surrounding late previtellogenic oocytes of *Karoophasma biedouwensis*, the nuclei exhibited a highly lobate (C-shaped) form, as seen in the webspinner *Oligotoma* (Niwa, 1994) and in the phasmid *Carausius* (Pijnacker and Godeke, 1984). It may be associated with polyploidization of nuclei probably to form proteins to be transported into the oocytes through the interdigitated microvilli. It is highly probable that the proteins are related to the formation of complex eggshell structures such as opercula or cap structures found in these orders (Mazzini *et al.*, 1993; Niwa *et al.*, 1993; Klass *et al.*, 2002; Zompro *et al.*, 2002; Machida *et al.*, 2004; Tsutsumi *et al.*, 2004).

As the sister groups of Mantophasmatodea, two orthopteroid orders have so far been designated: Phasmatodea by external morphology (Klass *et al.*, 2002) and Mantodea by sperm ultrastructure (Dallai *et al.*, 2003). However, the ovarian characteristics of *Karoophasma biedouwensis*, such as ovariole alignment and the emergence period of the organelles related to vitellogenesis, differing from those of Phasmatodea and Mantodea, respectively, do not support the sister group relationships between Mantophasmatodea and these two candidates. On the other hand, these ovarian characteristics seem to support a close affinity between Mantophasmatodea and Grylloblattodea, which has also been proposed by comparative embryology (Machida *et al.*, 2004), comparative ultrastructural study of egg membranes (cf. appendix of Tsutsumi *et al.*, 2004; Uchifune and Machida, 2005) and molecular evolutionary analyses (*e.g.*, Jarvis and Whiting, 2003) (cf. Gullan and Cranston, 2005). Further ultrastructural studies of ovarian characteristics of Mantophasmatodea, particularly on the vitellogenesis and the larval development of ovaries, may be needed to argue its phylogenetic position within orthopteroid orders.

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- Fig. 2 TEM showing the ovariole envelopes, follicular epithelium and periphery of early previtellogenic oocyte of *Karoophasma biedouwensis*. Note that the ovariole is covered with a thin non-cellular tunica propria (TP) and a thick cellular tunica externa (TE). Arrows indicate rough endoplasmic reticulum. Inset: enlargement of rough endoplasmic reticulum in the cytoplasm of tunica externa cell. FC: follicular cell, N: follicular cell nucleus, Oc: oocyte. Scales = 1  $\mu$ m.
- Fig. 3 TEM showing the germarium of adult ovary of *Karoophasma biedouwensis*. No intercellular bridges are formed between germ cells. Asterisks indicate remarkably flattened prefollicular cells. Oc: oocyte, Og: oogonia, TE: tunica externa. Scale = 1  $\mu$ m.
- Fig. 4 TEM showing the cytoplasm around the nucleus of middle previtellogenic oocyte of *Karoophasma biedouwensis*. Nuage material (NM), variously shaped mitochondria (Mt), rough endoplasmic reticulum (RER), Golgi apparatuses (asterisks) and small vesicles (arrows) derived from endoplasmic reticulum are observed. N: nucleus. Scale = 1  $\mu$ m.
- Fig. 5 TEM showing the peripheral region of late previtellogenic oocyte and follicular epithelium of *Karoophasma biedouwensis*. Interdigitation of microvilli (Mv) is formed between oocyte (Oc) and follicular cells (FC). Arrows show the deeply hollowed follicular cell nucleus (N). There are numerous pinosomes (arrowheads). TP: tunica propria. Scale = 1  $\mu$ m.

Table 1 Characteristics of ovarioles in Mantophasmatodea and other orthopteroid groups.

Order	Ovariole type*	Ovariole alignment	Ovariole number per each ovary	Ovariole envelope	References
Mantophasmatodea	panoistic	comb-like	5, 6	present	present study
Grylloblattodea	panoistic ?	comb-like	14	unknown	Matsuzaki <i>et al.</i> (1979)
Blattodea	panoistic ?	bunchy	6–20	present	Anderson (1964), Matsuda (1976), Büning (1994)
Mantodea	panoistic ?	bunchy	7–38	unknown	Matsuda (1976), Ogi and Iwaikawa (1980), Kanamaru (2002)
Isoptera	panoistic ?	bunchy	6–3, 200	present	Matsuda (1976), Grandi <i>et al.</i> (1988), Grandi <i>et al.</i> (1999), Büning (1994)
Phasmatodea	panoistic	comb-like	16–39	unknown	Matsuda (1976), Taddei <i>et al.</i> (1992, 1993), Büning (1994)
Embioptera	panoistic	comb-like	4, 5	present	Matsuda (1976), Niwa <i>et al.</i> (1993), Niwa (1994)
Orthoptera	panoistic	comb-like, bunchy, ramified	2–201	present	Matsuda (1976), Büning (1994)
Plecoptera	panoistic with germ cell cluster	comb-like	30–300	absent	Gottanka and Büning (1990), Murayama <i>et al.</i> (1995), Büning (1998)
Zoraptera	panoistic ?	comb-like	4–6	unknown	Matsuda (1976), Büning (1994)
Dermaptera	polytrophic meroistic	comb-like	5–30	present	Yamauchi and Yoshitake (1982), Büning (1994)

\* The ovariole types indicated by question marks have not been sufficiently investigated.

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